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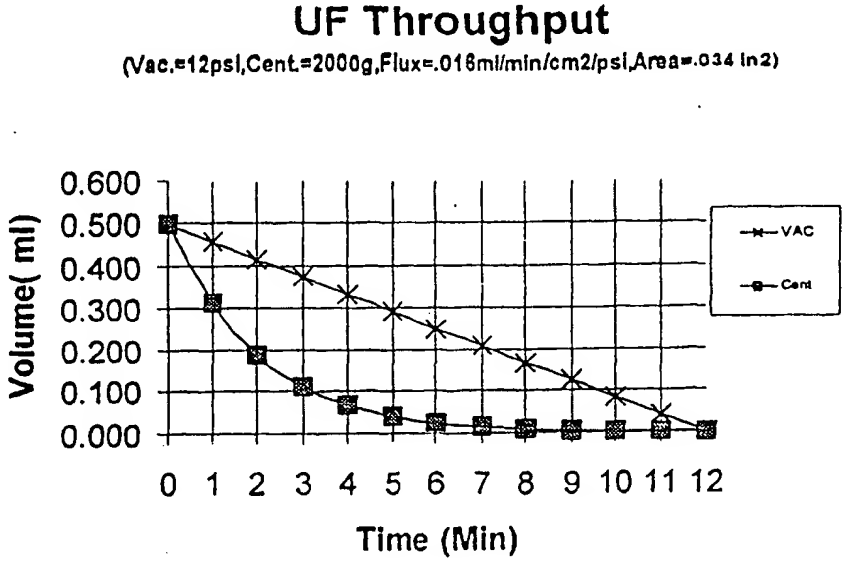
(54) Title: METHOD OF ULTRAFILTRATION



(57) Abstract

A process for ultrafiltration using constant pressure differential as the driving force is disclosed. This process is particularly suited for use in concentrating or purifying proteins and/or nucleic acids, often without any need for one or more diafiltration steps. The process is particularly suited for small volume applications, such as small concentrator devices and multiple well plates that typically use starting volumes of liquids of less than about 500 microliters. The steps include adding a liquid volume above an ultrafiltration membrane and applying a constant pressure differential at a force and length of time to achieve the desired concentration on the upstream side of the membrane. The concentrate is then diluted or removed for further processing.

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METHOD OF ULTRAFILTRATION

Related Cases

This case is related to USSN 60/132,369, filed May 5, 1999,entitled "Method of Ultrafiltration, designating Jack T. Leonard and Steve Toth as co-inventors.

The present invention relates to a method of ultrafiltration. More particularly, it relates to a method for ultrafiltration using constant pressure differential as the driving force.

Background of the Invention

Methods for ultrafiltration have relied on centrifugal forces to force the liquid component through an ultrafiltration membrane. Typically, positive pressure has been used when large volumes of fluid are to be filtered (e.g. stirred cells or spiral UF cartridges). Centrifugal force is the prevalent method used with small ultrafiltration devices, although some positive pressure attempts have been made with varying and impracticable results.

Ultrafiltration with small devices is becoming the staple in biological research such as DNA and protein research as researchers look to use smaller and smaller quantities in their research and as automation becomes more accepted. Such devices are either a single filter device such as is shown in US Patents 4,632,761, 4,772,972 and 4,832,851 or multiple well plates as is known from US Patents 5,141,718 and 5,223,133 .

The process of ultrafiltration in such devices is used to fractionate different sized molecules from each other. This may be used to remove impurities from the process used to develop or separate out the desired component or to separate the desired component from any other components in the liquid. The process results in a concentration of a retained solute above the membrane with a low molecular weight component passing through the membrane thereby removing it from the fluid being filtered. The liquid/ solid material retained above the membrane is called the retentate and the liquid below the membrane is called the filtrate.

In order to the remove or reduce the concentration of impurities such as low molecular weight components and various salts in the retentate, the material must be diluted by the addition of a liquid. While trying to affect a fractionation, low concentration factors sometimes delivered by

centrifugal ultrafiltration requires the use of multiple dilution and filtration steps (i.e. diafiltration) to deliver the retentate in a concentrated and sufficiently pure and relatively salt-free form. This is particularly true for small volume devices because of the low head height associated with these small volumes which results in low driving pressures. Moreover in the centrifugal process, the pressure doesn't remain constant. Rather it is always and continuously decreasing during the process as the volume and thus the head height decreases during filtration in that the pressure is determined solely by the height of the liquid column above the membrane and the g-force applied to the membrane face. However as the centrifuge process proceeds and the head height lessens, the pressure declines dramatically, resulting in flux decay (reduction in filtration rate and efficiency) over time.

Additionally, acceptable designs for centrifugal ultrafiltration devices and filtrate retrieval components are constrained by the centrifuge rotor dimensions and the strength of the plastics. These constraints, in turn, limit the maximum g-force that a given device can tolerate before failing. Ultimately, the membrane type and active membrane area, strength of the plastic housing, g-force, and the ability to achieve and maintain adequate head height will determine the performance of the centrifugal ultrafiltration device.

Vacuum manifolds are well accepted and widely used in many devices using microporous or macroporous filters. Positive pressure manifolds have also been suggested for microporous applications. However, ultrafiltration devices have not been designed to fit either manifold.

Moreover, there is a widely held belief in the art that ultrafiltration with vacuum is far too slow to be practical. In fact, this perception is held even for larger ultrafiltration devices where initial volumes are high for a fixed surface area of ultrafiltration membranes. This has led to the state of the art where centrifugal force is used to practice ultrafiltration of small samples.

US Patent 5,679,310 suggests using a vacuum to filter a multiple well plate which utilizes ultrafiltration or microporous hollow fibers or other large surface area configurations of membranes instead of flat sheet membranes. The hollow fibers are reported to be used in lieu of flat sheet membranes in order to increase the total surface area of the membrane over that available with a flat sheet. To the knowledge of the inventors, this product has never been commercialized.

The device has several drawbacks. First, it uses a highly three dimensional filter (hollow fiber) to increase overall surface area, but then severly restricts the ability of the filter to discharge filtrate from the well through the use of one or more small openings which connect the hollow fiber

lumen to the exterior of the well. The extremely small surface area available for filtration from the well as compared to a flat sheet (estimated as being less than about 20% of that of a flat sheet), results in a filtration rate that would not be close to that achieved with centrifugation on a flat sheet. Perhaps this is why vacuum was suggested, as a means for increasing the rate to a level closer to that of a flat sheet with centrifugation. Second, due to its 3-dimensionality, recovery of retentate from the well using conventional techniques such as pipetting is difficult, if not impossible. Last and most importantly, as the level of fluid in the well decreases, the flux and available filtration area also dramatically decline as less and less filter area is below the fluid level and thus rendering it progressively less capable of filtering the remaining fluid, leading to the same flux decay problems found with flat sheet devices using centrifugation.

What is desired is a process which overcomes the problems of centrifugation in flat sheet ultrafiltration or vacuum in 3-D hollow fiber ultrafiltration especially in small volume single or multiple well devices and which provides such filtration in a time comparable or shorter than that achieved with the centrifugation process today. The present invention provides such a process.

Summary of the Invention

The present invention provides a process for ultrafiltration using a constant pressure differential as the driving force for the filtration process. High flow rates can be maintained for durations sufficient for most applications using this process. This allows for separation of low volumes of starting materials in a shorter period of time than can be achieved with centrifugation. Additionally, constant pressure differential driven ultrafiltration is not subject to flux decay over time with non-polarizing solutes as occurs in centrifugal ultrafiltration. The consequence of either process is that much higher concentration factors can be achieved using constant pressure differential driven ultrafiltration as compared to centrifugal ultrafiltration. Additionally, although flux decay is observable using constant pressure differential driven ultrafiltration with polarizing solutes such as concentrated proteins, constant pressure differential driven ultrafiltration is faster than centrifugal ultrafiltration in most situations. Lastly, the process of constant pressure differential driven ultrafiltration reduces or eliminates the need for time-consuming repeat dilutions and filtrations that are frequently required with centrifugation to remove low molecular weight contaminants.

The present process involves taking an ultrafiltration device containing one or more ultrafiltration wells, each well containing an ultrafiltration membrane and applying a constant pressure differential to the liquid within the device in order to cause a constant pressure differential driven ultrafiltration to occur. The constant pressure differential is applied at a force and for a time sufficient to achieve a desired level of ultrafiltration.

It is an object of the present invention to provide a process for ultrafiltration comprising the steps of providing a device containing an ultrafiltration membrane, said membrane having an upstream and downstream sides, said device having a first reservoir adjacent the upstream side of the membrane for holding a volume of liquid to be filtered and having a second reservoir or drain on the downstream side of the membrane for collecting liquid that is filtered from the sample through the membrane, placing a liquid volume into the first reservoir of the device, and applying a constant pressure differential force to the liquid at a force and time sufficient to cause substantially all of the liquid to pass from the first reservoir through the membrane to the second reservoir or drain.

It is another object of the present invention to provide a process for the concentration of nucleic acids and proteins comprising the steps of providing an ultrafiltration membrane having an upstream and a downstream side, said membrane having a molecular cutoff between 100 Daltons (0.1kDaltons(kD)) and 300kD, adding a volume of liquid containing a biological material selected from the group consisting of nucleic acids, proteins and blends thereof and subjecting the material to a constant pressure differential until a desired concentration of the biological material on the upstream side is reached.

These and other objects of the present invention will become obvious from the specification and appended claims below.

In The Drawings

Figure 1 shows a graph depicting the rate and overall time required to filter a given volume of sample by constant pressure differential and centrifugation.(500 μl)

Figure 2 shows a graph depicting the rate and overall time required to filter a given volume of sample by constant pressure differential and centrifugation.(300 μl)

Figure 3 shows a close up of a portion of the graph of Figure 2.

Figure 4 shows a graph depicting the rate and overall time required to filter a given volume of sample by constant pressure differential and centrifugation.(100µl)

Figure 5 shows a graph depicting the effect of increasing the constant pressure differential over that used in Figures 1-4.(40psi)

Figure 6 shows a graph of the results from Example 1 utilizing a negative constant pressure differential.

Figure 7 shows a graph with the results from Example 2 utilizing a positive constant pressure differential.

Detailed Description of the Invention

The present invention is a process for the ultrafiltration and/or concentration of materials, preferably biological materials such as nucleic acids and/or proteins (although other materials typically filtered by ultrafiltration may be used).

The process involves applying a constant pressure differential force to the material to be filtered for a period of time and at a force sufficient to cause the desired level of filtration or concentration.

By " constant pressure differential " it is meant either a positive pressure or negative (or vacuum) pressure. Unlike the pressure of the centrifugal method in which the pressure is always decreasing over time due to a reduction in head height of the liquid, in a constant pressure differential process the pressure acting on the liquid can remain constant over the filtration cycle. Additionally, as the pressure is independent of head height of the liquid on which it is acting it may even be increased over time in order to drive the filtration process to completion. It is also within this definition to have a decrease in pressure over time if desired, however unlike in centrifugation, this decrease is controlled and is independent of head height of the liquid thus reducing or eliminating flux decay.

Ultrafiltration membranes are typically rated by their nominal molecular weight cutoff (ie the largest sized molecule which can pass through the particular membrane) rather than by average pore size (as with microfiltration membranes). Typically, molecular cutoffs in the range of from about 100 Daltons (100D) to about 500 kiloDaltons (500kD) are the preferred membranes useful in the present device. Preferably the range is from about 100D to about 300kD and more preferably

from about 3kD to about 300 kD. Ultrafiltration membranes can be made from a variety of materials including but not limited to polyamides, polysulphones, polyethersulphones polyarylsulphones, polyphenylsulphones, cellulosics, regenerated celluloses and polyvinylidene fluoride. They may be symmetrical or asymmetrical with asymmetrical designs being preferred.

One preferred method of the present invention is to use a small volume UF filtration device such as a single well device like Ultrafree® or Centricon® or Microcon® single well device available from Millipore Corporation, see U.S. Patent 4,632,761. These devices are designed for centrifugal filtration and are simply inserted into a centrifuge that is spun at certain revolutions to give a g-force sufficient to drive the ultrafiltration. In the present method, a constant pressure differential force is applied to the liquid in the device and the constant pressure differential becomes the driving force for the filtration process rather than the traditional g-force of centrifugation. When using a positive pressure differential (positive pressure), it is typically applied to the topside of the liquid to drive the liquid through the membrane. When a negative pressure differential or vacuum is used, the pressure is typically applied to the downstream side of the membrane so as to act on the bottom of the liquid and draw it through the membrane.

The same process can be used with a multiple welled plate, such as is disclosed in U.S. Patents 4,902,481 and 5,047,215.These plates are commercially available in a number of configurations, generally containing from about 6 to 1536 wells per plate. The most common plate has 96 wells and is available from Millipore Corporation as the MultiScreen® 96 well plate. In the process according to the present invention, the plate can be mounted on top of a vacuum manifold such that the vacuum is applied to the downstream side of the filter. A sample to be filtered is placed into one or more of the wells and the vacuum is applied at a force and time sufficient to reach the desired level of filtration. Alternatively a positive manifold can be mounted on the up-stream side of the membrane and a positive pressure differential can be applied to the liquid at a force and time sufficient to achieve the desired filtration.

The level of force (whether positive or negative) applied depends upon a number of factors among them are: the amount of sample to be filtered, the type of membrane used (the molecular cutoff of the membrane, its strength and thickness), the active filtration area of the membrane, the speed at which the filtration is to occur and the level of polarization of the sample.

The strength of the plastic is a much lesser variable especially as compared to centrifugal devices where plastic strength of the device in large part controls the g-force that can be supplied

and therefore the rate and amount of filtration which can be achieved. These means that thinner, less robust devices may be used and achieve comparable or superior filtration rates and percentages. One such advantage is the ability to use vacuum-formed trays especially with a vacuum driven or low positive pressure driven pressure differential process, something that is not available with centrifugal operated devices where such trays cannot withstand the required g-force for filtration to occur.

Additionally, unlike centrifugal filtration, constant pressure differential filtration is completely independent of the ability to achieve and maintain a head height meaning that the process is not typically subject to any flux decay at non-polarizing concentrations of solute. Typically, with small volumes, the consequence is that much higher concentration factors are achievable with constant pressure differential driven ultrafiltration as can be achieved with centrifugation within the same amount of time.

Normally, the volumes of liquid in which this process can be used will vary, with a high value of about 2 milliliters. More typically, it is used with volumes of less than 1 milliliter and preferably below 0.5 milliliters (500 microliters). This is clearly shown in the graph of Figure 1. As can be clearly seen there is an upper limit at which, due to sufficient head height, centrifugation is just as quick as the constant pressure differential process of the present invention (the exact level is dependent among other things upon the fluid used and the level of constant pressure differential and centrifugation used). However, as will be explained below in conjunction with diafiltration, even when the rates are clearly faster for centrifugation, there are other compelling reasons for still using the process of the present invention rather than centrifugation as it eliminates or lessens the need for diafiltration.

At lower volumes, it is clear however that use of the constant pressure differential of the present invention is clearly faster than that of centrifugation. The point at which the constant pressure differential process is faster than centrifugation is hereinafter referred to as the "breakthrough point". Figure 2 shows just such an example. Here, the breakthrough point is at about the 7 minute mark. As shown in Figure 2, when using a small volume, about 0.300 milliliters, the present process is about 60% faster than centrifugation.

Figure 3 is a close up of the graph of Figure 2 for the last 0.020 milliliters of fluid to be filtered. Here the differences between the present invention and centrifugation and the breakthrough point can be clearly and dramatically shown.

Figure 4 shows the same effect with even smaller starting volumes of fluid. Here, as shown, the present process is about 5 times faster that of centrifugation.

Figure 5 shows the effect that varying the level of the constant pressure differential has on the process. In this example, a 3.5 fold increase in the constant pressure differential over that applied in Figure 1 results in an almost 6 fold increase in filtration speed. Additionally, the breakthrough point occurs at about 1 minute as compared to 7 minutes for that of Figure 1.

Flux decay may occur in filtering materials that have a high level of polarizing characteristics. In those instances, some flux decay may be observed during filtration by the present process, but this is independent of head height and has to do with the inherent properties of the material being filtered. This means that smaller starting quantities of sample may be used and high levels of ultrafiltration and recovery can be achieved at satisfactory rates even with the presence of such polarizing materials, something that is not always possible with centrifugal processes.

The constant pressure differential may be negative, e.g. a reduced pressure(below atmospheric) or a vacuum or positive e.g. above atmospheric.

Typically, a negative pressure differential or vacuum force of from about 5 inches Hg to about 27 inches Hg can be used (169-914 millibars) More preferably from about 10 to about 27 inches (338-914 millibars) can be used. The level of vacuum force can be easily varied by the user to fit the desired parameters of the system, the rate of ultrafiltration desired and the sample he/she is using.

Typically, a positive pressure differential of from about 5 to about 80 psi can be used. Higher pressures may be used with devices that have the strength to withstand such pressures. More preferably, from about 40 to about 60 psi can be used. The level of positive pressure can be easily varied by the user to fit the desired parameters of the system, the rate of ultrafiltration desired and the sample he/she is using.

The amount of starting fluid to be filtered can vary widely. However this process has been found particularly useful with small volumes of liquid which cannot typically generate or maintain a suitable head height. Such volumes are generally under about 1000 microliters, preferably less than about 500 microliters and may be as little as 1 microliter.

An additional advantage of the process of the present invention is that the need for diafiltration (reduction of salts or contaminants by repeated dilutions in ultrapure water or solvent

followed by centrifugal filtration to remove the solvated impurities and salts) can be reduced or eliminated, making this process of particular benefit to the biological research area where such diafiltration steps are time consuming and if not complete, can skew the results obtained.

It has been known that a single pass centrifugation process will not remove all salts and other impurities from a biological sample. Therefore, the normal protocol is to dilute the retentate in ultrapure water or a solvent and re-centrifuge the material one or more times in order to draw out a sufficient volume of these impurities.

It has been discovered that in normal centrifugation, as the volume of liquid above the membrane gets below a certain level, typically below 1 microliter volume, evaporation of the liquid is primarily phenomenon responsible for the removal of the liquid, not ultrafiltration. This is due to the low head height that results in little if any pressure being applied to the remaining liquid and thereby little if any filtration occurring. Because of this, any impurity is simply dehydrated onto the surface of the filter. When a reconstituting liquid is added to the retentate, these materials simply dissolve into the liquid and remain with the retentate. This explains the need for several diafiltration steps.

It has been discovered that in using the constant pressure differential process of the present invention filtration remains the dominant means for removing these impurities (as it is independent of head height in order to function), thereby causing the impurities to pass through the membrane and out of the retentate to a much greater level than can be achieved with centrifugation. Essentially all impurities are removed with the current process in a single pass whereas often less than 90% of all impurities are removed with a single pass using the traditional centrifugation process. This allows one to reduce or to eliminate the need for diafiltration steps after filtration and provides a purer product for further use.

As mentioned above, this process is particularly useful when starting with small volumes as the process is quicker than centrifugation. Additionally when the desire is to remove impurities from a biological sample, this process may be used with larger starting samples even though the filtration time may be longer than that for a centrifugation process as it will result in a purer retentate with fewer if no diafiltration steps. The overall time savings (filtration and diafiltration) can justify the apparent increase in filtration time.

Alternatively, one may combine the efficiencies of both processes to process large volumes of biological samples with higher resultant purity. One may simply centrifuge the initial

volume until about 20% or less of the original volume remains (or about at least 80% of the initial volume has been removed by centrifugation). Thereafter, one applies the constant pressure differential process of the present invention to remove remaining impurities from the sample. In doing so, one can substantially reduce the time needed to conduct the ultrafiltration as well as reduce or eliminate the need for several diafiltration steps.

Example 1

One hundred microliters of distilled water were placed in all wells of 96-well ultrafiltration plates with a nominal molecular weight cut-off of 30 kD. The plate was placed on top of a collection plate and it was centrifuged at 2,000 x g in a swinging bucket rotor. Plates were also placed on a vacuum manifold, and a vacuum applied at 24 inches of Hg (12 psi), and the time required to "go to dryness" was determined. The amount of solution remaining in the plates was determined by weighing on a microbalance after processing for specific time intervals.

The average amount of solution remaining per well was plotted as a function of time (Figure 6). This figure illustrates that higher concentration factors are achievable by vacuum under these conditions, and that the experimental values closely fit the predicted values.

Example 2

Centricon ® centrifugal ultrafiltration units with a nominal molecular weight cut-off of 30 kD were each loaded with 1.64 milliliters of distilled water. The Centricon® units were centrifuged at 2,000 x g in a swinging bucket rotor. Another set of Centricon® units were loaded with 1.64milliliters of water and 40 psi of constant positive pressure was applied to the reservoir using compressed air. The amount of solution remaining in the Centricon® was determined by weighing on a microbalance after applying constant positive pressure, or after centrifuging for specific time intervals. Retained liquid was recovered from the centrifuged Centricon® units using the patented reverse spin technique.

The average amount of solution remaining per Centricon® unit was plotted as a function of time (Figure 7). Theoretical values, calculated using known flow rate for this membrane type, are also plotted. This Figure illustrates that higher concentration factors are achievable by constant positive pressure under these conditions, and that the experimental values for both positive

pressure and centrifugation closely fit the predicted values. Even after 17 minutes of centrifugation. 2.5 microliters on average could be recovered from the Centricon® units by reverse spinning. This provides evidence that centrifugal ultrafiltration is indeed asymptotic (*i.e.*, does not achieve zero volume by active ultrafiltration).

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What I Claim:

Claims:

1) A process for the concentration or purification of nucleic acids and proteins comprising the steps of providing an ultrafiltration membrane having an upstream and a downstream side, said membrane having a molecular cutoff between 100 D and 300kD, adding a volume of liquid of less than about 1000 microliters, said volume containing a biological material selected from the group consisting of nucleic acids, proteins and blends thereof and subjecting the volume to a constant pressure differential until a desired concentration of the biological material on the upstream side is reached.

2) A process for the concentration or purification of nucleic acids and proteins comprising the steps of providing an ultrafiltration membrane having an upstream and a downstream side, said membrane having a molecular cutoff between 100 D and 300kD, adding a volume of liquid containing a biological material selected from the group consisting of nucleic acids, proteins and blends thereof and subjecting the material to centrifugation until at least about 350 microliters or less of the initial volume remains, then subjecting the remaining volume to a constant pressure differential until a desired concentration of the biological material on the upstream side is reached.

3) The process of claim 1 wherein the constant pressure differential is a vacuum from about 169 millibars to about 914millibars

4) The process of claim 2 wherein the constant pressure differential is a vacuum from about 169 millibars to about 914millibars

5) The process of claim 1 wherein the constant pressure differential is a positive pressure from about 5 to about 80 psi.

6) The process of claim 2 wherein the constant pressure differential is a positive pressure from about 5 to about 80 psi.

7) The process of claim 1 wherein the device is a single well device.

8) The process of claim 1 wherein the device is a multiple well device.

9) The process of claim 1 wherein the device is a 96 well plate.

10) The process of claim 1 wherein the number of wells in the plate is from about 6 to about 1536.

11) The process of claim 2 wherein the device is a single well device.

12) The process of claim 2 wherein the device is a multiple well device.

13) The process of claim 2 wherein the device is a 96 well plate.

14) The process of claim 2 wherein the number of wells in the plate is from about 6 to about 1536.

15) The process of claim 1 wherein the membrane has a molecular cutoff of from about 100 Daltons to about 500 kDaltons.

16) The process of claim 2 wherein the cutoff is from about 3 to about 300 kDaltons.

17) The process of claim 1 wherein the membrane is made of a material selected from the group consisting of polyamides, polysulphones, polyethersulphones polyarylsulphones, cellulosics, regenerated celluloses, polyolefins such as polyethylene and polypropylene and polyvinylidene fluoride.

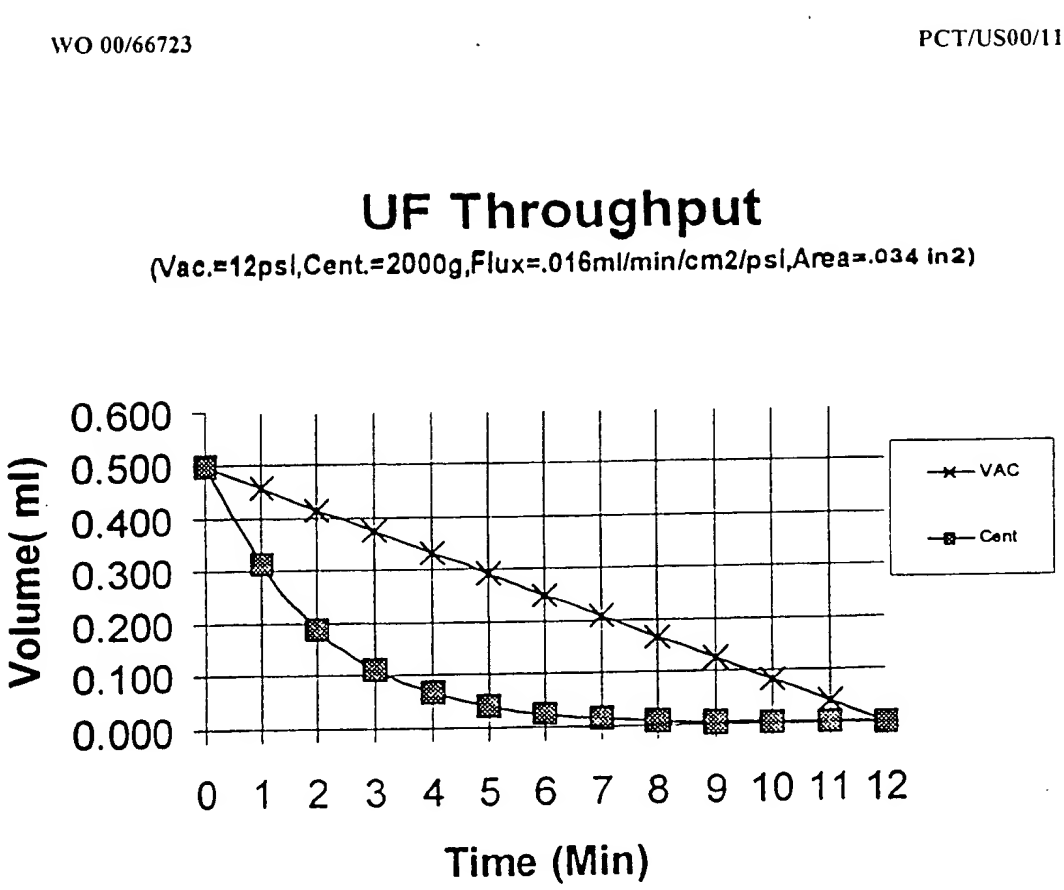
18) The process of claim 2 wherein the membrane is made of a material selected from the group consisting of polyamides, polysulphones, polyethersulphones polyarylsulphones, cellulosics, regenerated celluloses, polyolefins such as polyethylene and polypropylene and polyvinylidene fluoride.

19) The process of claim 1 wherein the starting volume of liquid is less than about 500 microliters.

20) The process of claim 1 wherein the starting volume of liquid is less than 350 microliters.

21) The process of claim 1 wherein the process is free of a diafiltration step.

22) The process of claim 2 wherein the process is free of a diafiltration step.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0.300
0.200
0.100
0.000
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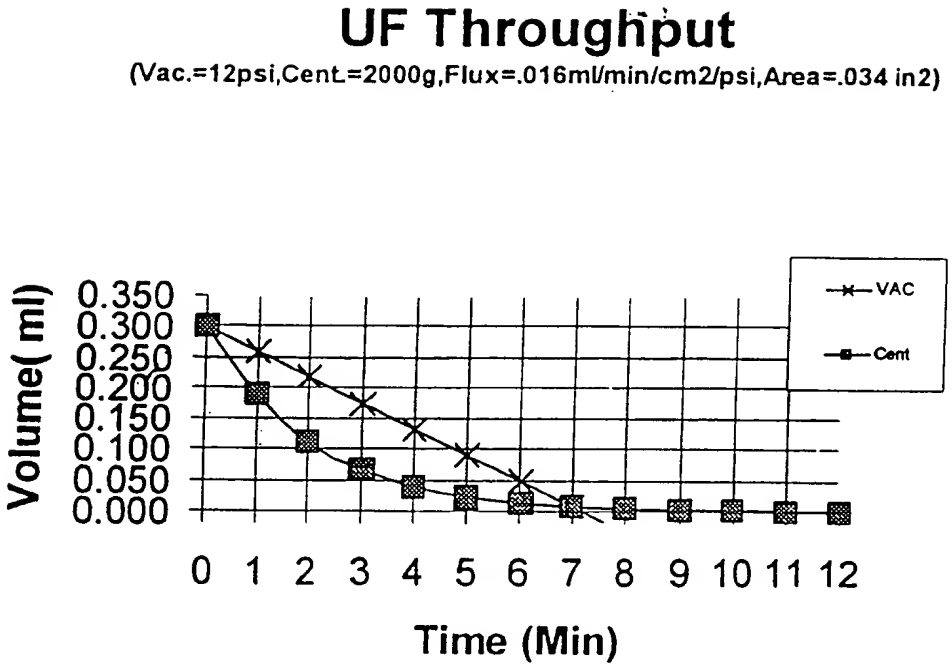
Time (Min)
VAC
Cent

Figure 1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Time (Min)
VAC
Cent

Figure 2

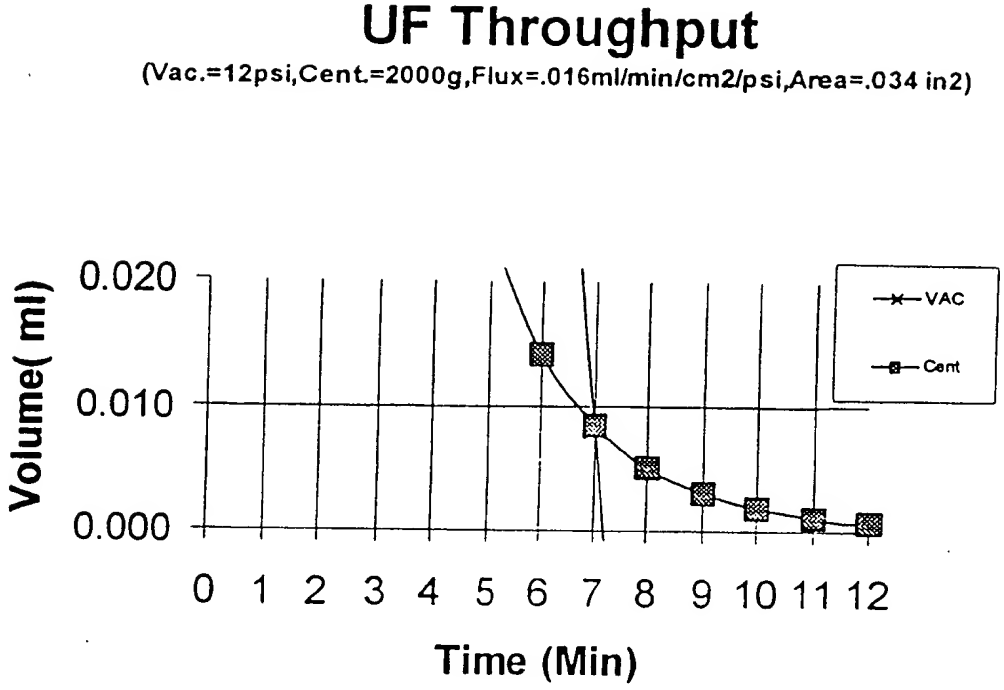
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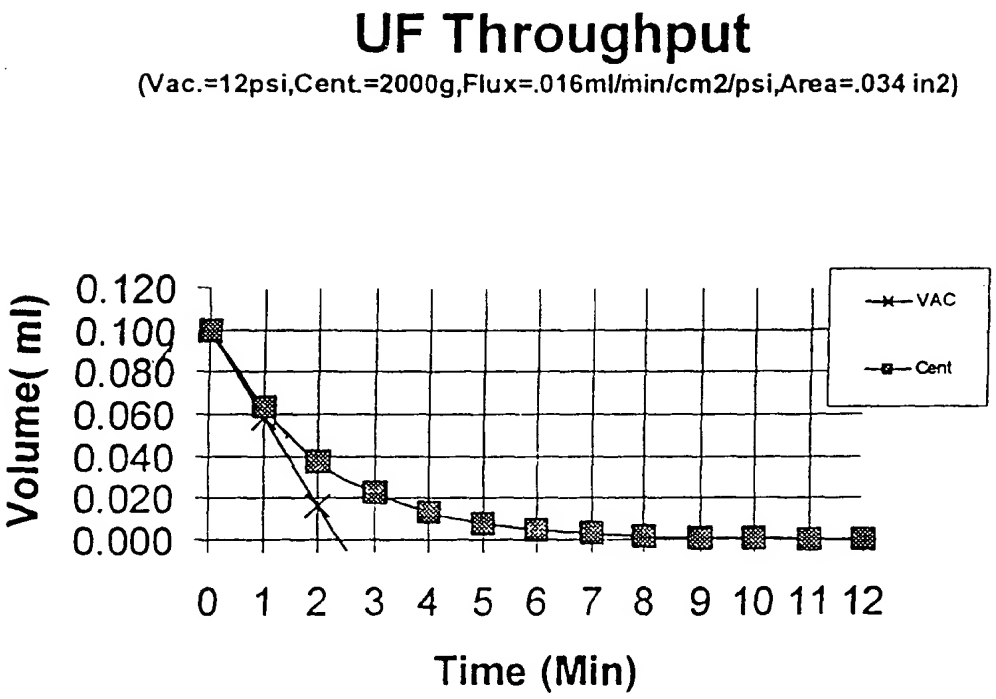
Figure 3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0 1 2 3 4 5 6 7 8 9 10 11 12
Time (Min)
VAC
Cent

Figure 4

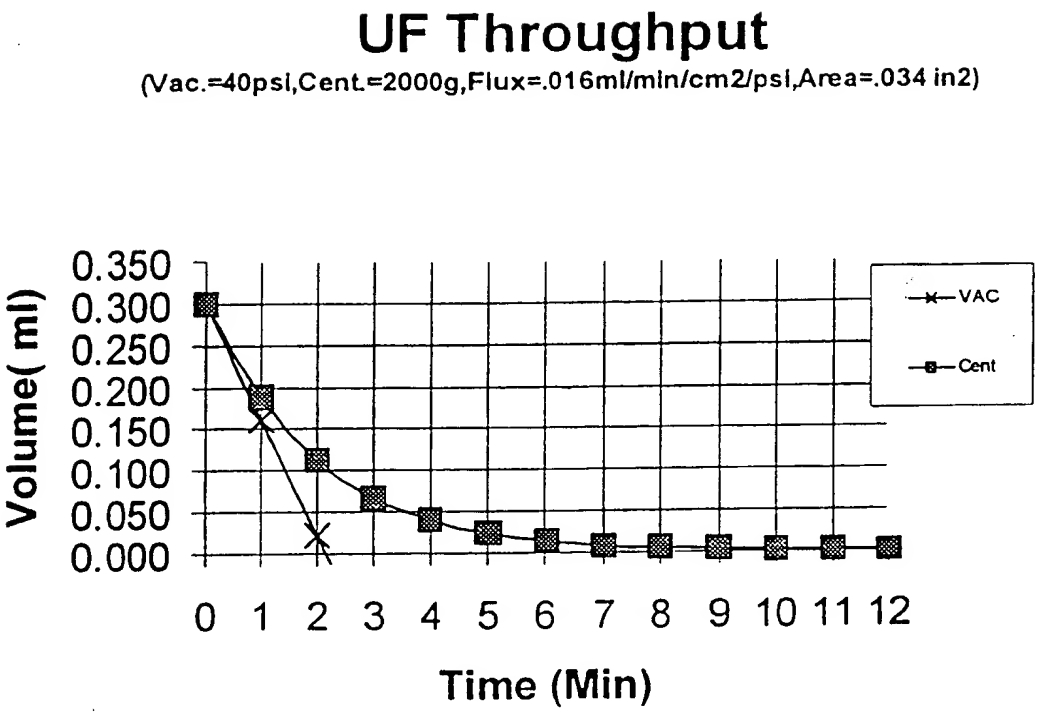
UF Throughput
(Vac.=40psi,Cent.=2000g,Flux=.016ml/min/cm2/psi,Area=.034 in2)
Volume(ml)
0.350
0.300
0.250
0.200
0.150
0.100
0.050
0.000
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (Min)
VAC
Cent

Figure 5

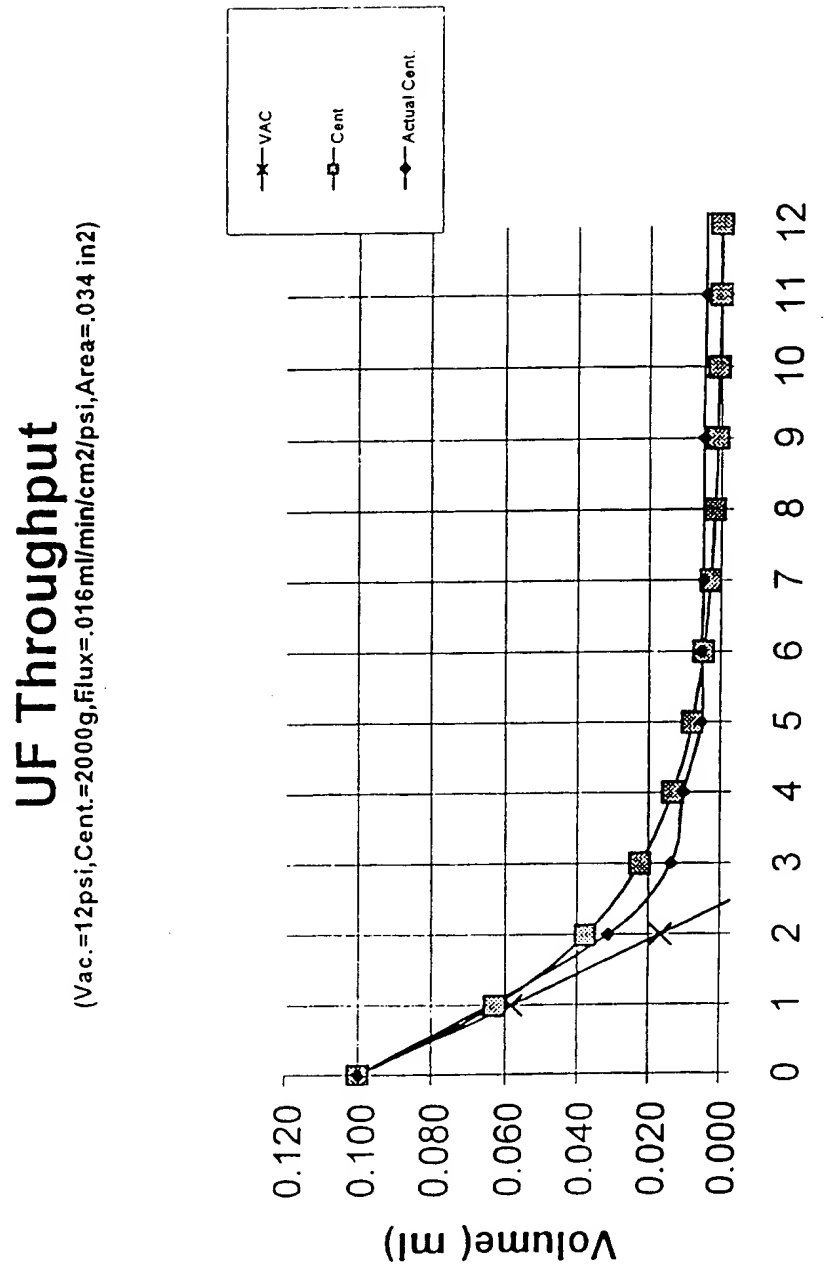
UF Throughput
(Vac.=12psi,Cent.=2000g,Flux=.016ml/min/cm2/psi,Area=.034 in2)
VAC
Cent
Actual Cent.
Volume(ml)
0.120
0.100
0.080
0.060
0.040
0.020
0.000
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (Min)

Figure 6

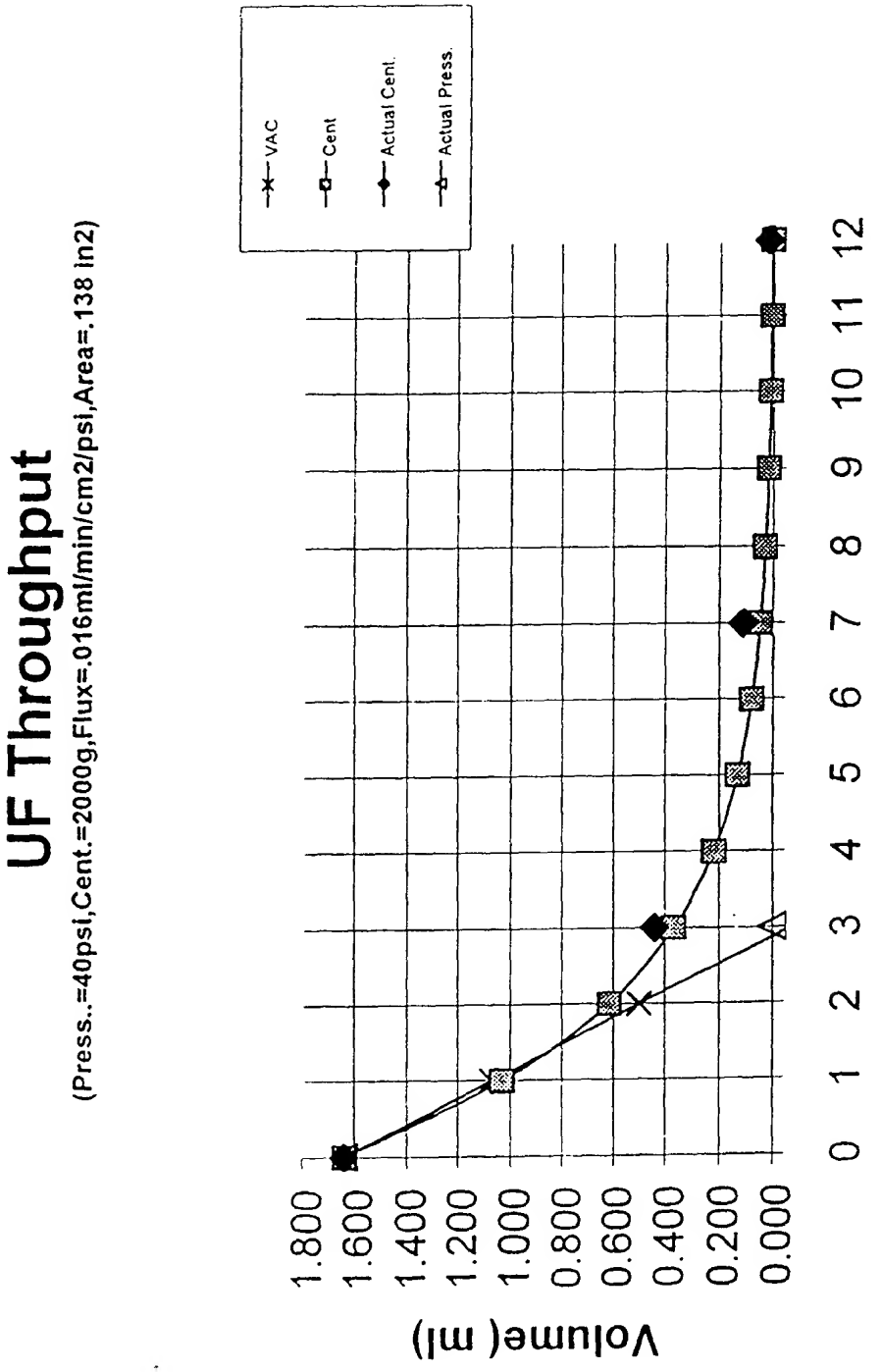
UF Throughput
(Press..=40psi,Cent.=2000g,Flux=.016ml/min/cm2/psi,Area=.138 in2)
VAC
Cent
Actual Cent.
Actual Press.
Volume(ml)
1.800
1.600
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0 1 2 3 4 5 6 7 8 9 10 11 12
Time (Min)

Figure 7

INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/11926

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/10 C07K1/34 B01D61/14

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS, FSTA, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 13963 A (HYMAN EDWARD D) 20 August 1992 (1992-08-20) * the whole document, in particular example 5, pages 15-16 and figures 1 and 4 * ---	1,8,10, 15,21
X	US 4 690 754 A (KOYAMA KENJI ET AL) 1 September 1987 (1987-09-01) the whole document ---	1,7,15, 17,19-21
X	WO 87 07645 A (LONDON HOSPITAL MED COLL) 17 December 1987 (1987-12-17) the whole document ---	1,7,15, 17,21
A	EP 0 431 905 A (TOSOH CORP ;RIKEN INST OF PHYSICAL AND CHE (JP)) 12 June 1991 (1991-06-12) the whole document --- -/--	1,2, 7-10,17

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
25 August 2000	12/09/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Oderwald, H

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INTERNATIONAL SEARCH REPORT

Inter onal Application No
PCT/US 00/11926

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	M.C. LINHARES AND P.T. KISSINGER: "Capillary ultrafiltration : in vivo sampling probes for small molecules" ANAL. CHEM., vol. 64, 1992, pages 2831-2835, XP000938635 the whole document	1,3,15, 17,19-21

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 00/11926

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9213963 A	20-08-1992	NONE	
US 4690754 A	01-09-1987	JP 61209010 A	17-09-1986
		DE 3608062 A	09-10-1986
		GB 2173711 A,B	22-10-1986
WO 8707645 A	17-12-1987	EP 0268647 A	01-06-1988
		JP 1500482 T	23-02-1989
EP 0431905 A	12-06-1991	JP 2978518 B	15-11-1999
		JP 3180182 A	06-08-1991
		DE 69008825 D	16-06-1994
		DE 69008825 T	25-08-1994